Identification of vectors of cocoa swollen shoot virus and control methods: A perspective from Côte d’Ivoire

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1. BACKGROUND

2. CURRENT STATUS OF KNOWLEDGE
   - Morphological identification of mealybugs
   - Molecular identification of mealybugs
   - Detection on virus into mealybugs
   - Control of CSSV vectors

3. DIFFICULTIES

4. CONCLUSION

5. PERSPECTIVES
1. BACKGROUND
It is known that CSSV is transmitted by mealybugs (Family: Pseudococcidae).

At least 14 species of mealybugs are known as vectors of CSSV;

Previous works on mealybugs control were difficult:

- Some chemicals non-effective or those which are effective are also toxic
- Works on biological control are done in some countries like Ghana but there is not yet application in fields
2. CURRENT STATUS OF KNOWLEDGE
MORPHOLOGICAL IDENTIFICATION OF MEALYBUGS
Survey in cocoa orchards and identification:

- Trees random selected per field;
- Looking for mealybugs on pods, trunk, leaves, flowers, chupons;
- Identification based on external body of female adults;
- Counting all colonies found;
- Identification in laboratory of non-identified specimens in fields using binocular magnifying.
Morphological identification of mealybugs

3 Species which do not belong to Pseudococcidae family

*Stictococcus gowdeyi* *(Stictococcoidae)*
*Ceroplastes spp.* *(Cocoidae)*
*Stictococcus sjöstedti* *(Stictococcoidae)*
Morphological identification of mealybugs

7 Species of Pseudococcidae family

- **Formicococcus (Planococcoides) njalensis**
- **Planococcus kenya**
- **Ferrisia virgata**
- **Phenacoccus hargreavesi**
- **Dysmicoccus brevipes**
- **Planococcus citri**
- **Pseudococcus longispinus**
MOLECULAR IDENTIFICATION OF MEALYBUGS
Molecular identification of mealybugs

- Sampling in fields

- Molecular analysis:
  - Extraction of mealybugs’ DNA;
  - PCR with MTD 10/12 primers to amplify mtCOI gene;
  - Agarose gel electrophoresis
  - Sequencing and analyze of sequences;
  - Building the phylogenetic tree.
Molecular identification of mealybugs

Migration of DNA after Agarose gel electrophoresis

✓ Good reaction: migration of DNA at 800 ~ 850 pb.

PCR Annealing Temp: 57°C
0.7% Agarose
1XTAE Buffer
120 V, 40 min
2.5 µL Sample
2.0 µL Ladder
Molecular identification of mealybugs

Phylogenetic tree of 47 mealybugs CI and 16 ref. genebank

**Formicococcus njalensis CI**

**Planococcus citri (Genebank)**
**Planococcus citri (CI)**

**Others mealybugs species (Genebank)**
Molecular identification of mealybugs

Phylogenetic tree of 47 mealybugs CI and 16 ref. genebank

- Zuénoula
- Abengourou
- Agboville / Adopé
- Buyo
- Bangolo / Duékoué
- Divo
- Aboisso

Planococcus citri (Genebank)
Planococcus citri (CI)
DETECTION OF CSSV INTO MEALYBUGS
Detection of CSSV into mealybugs

How long mealybugs are viruliferous?

- Feeding non viruliferous mealybugs on infected seedlings for 3 days in greenhouse

- Killing mealybugs with 75° ethanol

- DNA extraction, PCR and agarose gel electrophoresis
Detection of CSSV into mealybugs

Agarose gel electrophoresis

- Positive reaction of some samples: Y30mn, Y60mn, Y2h, Y6h, Y10h
- Migration of the positive control at the same size (~400 bp)
- Presence of CSSV into mealybugs.

0.7% Agarose, 1XTAE Buffer, 120 V, 40 min, 2.5 µL Samples, 2.0 µL Ladder.
CONTROL OF CSSV VECTORS
Chemical control

- New insecticides available and already used in mirids control (imidacloprid, acetamiprid, thiacloprid)
- Test the effectiveness of these insecticides on mealybugs
- Study the effect of these new insecticides on cocoa beans quality
3. DIFFICULTIES
Morphological identification is difficult:
- Adapted key of identification;
- Slide preparation;
- Need taxonomist to confirm.

DNA extraction not easy for all development stages of mealyubgs:

No sequence of *Formicococcccus (Planococccoides) njalensis* in genebank;
4. CONCLUSION
Morphological identification is difficult;

Techniques are available for molecular identification of mealybugs;

CSSV can be detected into mealybugs;

New systemic insecticides are available to be tested for the control of mealybugs.
5. PERSPECTIVES
PERSPECTIVES

- Improve the molecular technique for mealybugs identification;
- Determine the genetic diversity of mealybugs in Côte d’Ivoire;
- Continue detection of CSSV in different species of mealybugs in greenhouse and fields;
- Identify and control mealybugs hosts plants in cocoa orchards.
AKNOWLEDGMENTS
THANK YOU FOR
YOUR ATTENTION